

XIV. THE DIGESTION AND ABSORPTION OF PROTEIN AND FAT IN NORMAL AND DEPANCREATISED ANIMALS.

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Von Noorden [1893] and Rubner [1879] showed that on a meat or concentrated protein diet, the protein N was well absorbed, the utilisation figure varying from 90 to 98 per cent. These and several other workers have shown that within limits the greater the amount of protein given in a digestible form the better is its utilisation.

Within the limits of normal dietary there are several factors which influence the absorption of fat. Von Noorden's experiments [1907, 1] with butter fat given in varying amounts in an otherwise uniform diet show clearly that proportionately more fat is excreted when the food is almost fat free than when a great deal of fat is administered. With 4.2 g. fat in the food the utilisation was 42.9 per cent., while with 80.2 g. the figure rose to 93.6 per cent.

The physical consistency and the melting point of the fat are of importance as has been demonstrated by Munk [1884] and by Arnschink [1890], who have fully worked out the relation of the melting point to facility of absorption. Again, as Bucheim [1874] pointed out with regard to the ready utilisation of cod liver oil, the percentage of free fatty acid present in a fat exercises a marked influence. Blumfeld and Hauser [1888] obtained quite as good results with butter and milk fat as with preparations containing a certain percentage of free fatty acid.

As far back as 1851, Traube and others showed that it was possible in

diabetics to obtain a good utilisation of the food given. Hirschfeld [1891] in a paper on a new clinical form of diabetes stated that he could only recover 32 per cent. of the N ingested, while Brugsch [1906] found a loss of 20–25 per cent. of N and 50 to 60 per cent. of the fat ingested.

From a clinical point of view a considerable amount of work has centred around the question of the utilisation of fat in diabetes. E. Zunz and Mayer [1904] confirmed the statement that the closing of the pancreatic duct leads to a slowly ensuing disturbance of absorption. This impairment of function is slight compared with the disturbance following total extirpation with a diet containing no pancreas. In dogs, after total extirpation of the pancreas Abelmann [1890] found the following fat absorption figures: in small intestine non-emulsified fat, 32 per cent.; emulsified and milk fat, 80 per cent.; in the faeces on a milk diet there was a loss of 43–70 per cent. Müller [1887] and von Noorden [1907, 2] and others state that with disorders of pancreatic secretion it is the fat-splitting process which is affected. This view was opposed by Deucher, who obtained in severe disorders of the pancreas as complete a splitting of the fat as in the healthy organism, 62–80 per cent.

In the experiments described in this paper I have endeavoured to determine the amount of absorption which takes place in normal animals under a low and high protein and fat diet respectively, and to note what variation from the normal figures results when the animal is

- (1) partially depancreatized (a) with subcutaneous graft of pancreas,
(b) with small piece of pancreas *in situ*,
- (2) totally depancreatized, and
- (3) under these conditions to see what effect is produced upon absorption by the addition of raw pancreas to the diet.

Methods of fat extraction and estimation.

The total quantity of faeces is dried in an air oven at room temperature, the process occupying 12–20 hours. The dried faeces are powdered thoroughly till the whole sample readily passes through a fine sieve, after which the sample is again rubbed up in a mortar till the whole is reduced to a very fine powder, which is then weighed. One gram is accurately weighed out and transferred to a Jena flask, 20 cc. 20 per cent. NaOH added, and the whole heated in a water-bath for three hours as recommended by Kumagawa and Suto [1908]. The warm alkaline solution is then poured into a separating funnel and acidified with HCl; the acid is added in small quantities and after

each addition the separating funnel is well shaken under cold running water. When the whole is thoroughly cooled, about 50 cc. of ether are added and the mixture shaken at short intervals for a quarter of an hour and set aside for a few minutes to allow the solutions to stratify. This shaking is repeated three times and the separating funnel is then set aside for an hour so that all the ether may rise to the surface and a distinct thin film of demarcation between the two fluids become apparent. The lower layer is then separated, leaving the precipitate in the funnel, and the ether poured into a 200 cc. flask. The precipitate is dissolved in NaOH and shaken with ether; the separated acid layer is returned to the funnel and the whole extracted with ether twice. The ether is then evaporated, the residue redissolved in anhydrous ether and filtered through a capillary filter similar to that described by Mottram [1909]. The resulting solution is again evaporated, the residue dissolved in petroleum ether, allowed to stand for half an hour, and filtered. The petroleum ether is evaporated and the residue dried at 70° for 4–5 hours till of constant weight.

Folin and Wentworth [1910] have shown that everything taken out by organic solvents is not fat, and that it is impossible to determine separately free fatty acids and soaps. The ordinary fatty acids are very weak, and the extent to which they combine with bases to form soaps in faeces is not important. The equilibrium between soaps and fatty acids is influenced by so many factors, H_2S , CO_2 , NH_4 , heat, etc., during the drying, that the proportion of soaps, free fatty acids and neutral fat at the end of the process bears no relation to that existing at the time of defaecation. The new method described by Folin and Wentworth for the estimation of the fatty acids has been used. The solvent used was warm benzene and the dissolved fatty acid was titrated with N/10 alcoholic soda, phenolphthalein being used as an indicator. On several occasions the mean molecular weight of the fat of the faeces was determined, both with normal and diabetic samples, and under normal conditions of intestinal activity, and it was found to approximate closely to the molecular weight of stearic acid.

The estimation included the total fat, extracted as fatty acid plus cholesterol, lecithin and unsaponifiable substances present in the stool. The fatty acid was determined by the titration described above, while the difference between the fatty acid and the total fat extracted by petroleum ether was taken as an indication of the amount of lecithin and unsaponifiable substances in the faeces.

The N was determined by the Kjeldahl method.

Animals.

The faeces for the periods were demarcated by an addition of bones to the diet. The dogs were weighed at regular intervals, the diabetic animals being weighed every day. While under observation the dogs were kept in metabolism cages. They were fed usually once a day (about 5 p.m.), no food being left in the cages over night. The food was weighed and precautions taken against wasting of the weighed amount.

Samples of the food were analysed from time to time. The following table gives the percentage analysis:

| | | N | Fat |
|------------------------|----|-------------|------|
| Horse meat (cooked) .. | .. | 4.1 to 4.41 | 3.0 |
| Ox pancreas | .. | 2.89 | 12.1 |
| Caseinogen | .. | 15.0 | — |
| Puppy biscuit | .. | 3.14 | 3.48 |
| Suet | .. | 0.730 | 82.0 |
| Milk | .. | 0.52 | 3.5 |
| Ereptone | .. | 12.8 | — |

Operations.

All the operations were performed by Professor Starling. The dogs were given a small dose of morphia hypodermically about two hours previously and then the operation was carried out under CE anaesthesia.

In the case of Dog I where a graft of the gland was left, all the pancreatic tissue was removed from the abdominal cavity, a small portion of the tail of the gland with its blood supply intact through a pedicle of mesentery being placed under the skin of the abdominal wall. The graft was functional, secreting a clear fluid for a short period after the operation, which could slowly digest fibrin.

With regard to Dog II, which was also partially depancreatized, a small portion of the gland was left *in situ* close to the main pancreatic duct, which was ligated peripherally. After removal of this piece of pancreatic tissue, the dog, which had a reduced sugar tolerance, became markedly diabetic, but remained in good condition for some time.

THE UTILISATION OF PROTEIN N AND FAT IN NORMAL DOGS.

Table I gives the total and percentage excretion of N and fat and the percentage of fatty acid of total petroleum ether extract, which latter includes lecithin, cholesterol and unsaponifiable substances, etc.

| Date | | Dry wt. of Faeces g. | Total N | % N | Total fat | % fat | Total fatty acid | % fatty acid of total P. E. Extract |
|--|----|-------------------------------|---------|------|-----------|-------|------------------------|---|
| 1. Diet: puppy biscuit, 200 g.—5 days. | | | | | | | | |
| Dec. | 1 | 15 | 1.08 | 4.73 | 0.720 | 3.60 | — | — |
| | 3 | 20 | 0.870 | 4.62 | 1.038 | 7.42 | — | — |
| Weight, 10-15 kilos. | | | | | | | | |
| 2. Diet: meat, 250 g.—5 days. | | | | | | | | |
| | 10 | 15 | 0.693 | 4.48 | 2.01 | 13.04 | 1.260 | 60 |
| | 11 | 20 | 0.574 | 2.87 | 1.59 | 7.98 | 0.963 | 60.5 |
| | 12 | 14 | 0.300 | 2.10 | 0.670 | 4.78 | 0.371 | 56.7 |
| | 14 | 16 | 0.493 | 3.08 | 0.592 | 3.20 | 0.273 | 53.6 |
| Weight, 9.70 to 9.65 kilos. | | | | | | | | |
| 3. Diet: milk, 200 c.c.; puppy biscuit, 100 g.; meat, 100 g.—9 days. | | | | | | | | |
| | 18 | 17 | 1.05 | 6.23 | 1.67 | 9.80 | 0.91 | 54 |
| | 19 | 20 | 1.26 | 6.30 | 1.432 | 7.40 | 0.624 | 43.6 |
| | 20 | 23 | 1.16 | 5.04 | 1.45 | 7.80 | 0.759 | 52.1 |
| | 22 | 15.5 | 0.90 | 5.46 | 1.55 | 10.0 | 0.924 | 59.6 |
| | 24 | 24 | 1.377 | 5.74 | 1.69 | 7.0 | 0.824 | 48.8 |
| Weight, 10 to 9.6 kilos. | | | | | | | | |
| 4. Diet: milk, c.c. per diem, 700, 700, 650, 600, 350, 250, 400, 400, 400. Puppy biscuit, 89 g. per diem (7th and 8th); 90 g. for last 3 days (9th, 10th and 11th). | | | | | | | | |
| Jan. | 6 | 23 | 1.03 | 4.48 | 6.716 | 29.2 | 4.301 | 64.2 |
| | 8 | 18.4 | 0.773 | 4.06 | 1.884 | 10.24 | 0.889 | 50.2 |
| | 9 | 19.5 | 1.133 | 5.74 | 1.840 | 9.46 | 0.866 | 47.8 |
| | 12 | 30 | 1.470 | 4.76 | 1.530 | 5.14 | 0.766 | 56.8 |
| Weight, 10 to 10.1 kilos. | | | | | | | | |
| 5. Diet: milk, 400 c.c.; puppy biscuit, 90 g.; lemco, 4, 8, 12, 16, 30, 16, 16, 8 g. per day. | | | | | | | | |
| Feb. | 15 | 37.5 | 1.84 | 4.9 | 1.77 | 5.55 | 1.065 | 51.4 |
| | 17 | 22.5 | 1.230 | 5.45 | 1.07 | 4.35 | 0.639 | 65.1 |
| | 20 | 42.0 | 2.226 | 5.32 | 2.788 | 6.64 | 0.984 | 40.0 |
| Weight, 8.85 to 8.80 kilos. | | | | | | | | |
| 6. Diet: meat, 400 g. first 2 days; 750 g. next 3 days; ereptone, 134.5 g. | | | | | | | | |
| Mar. | 4 | 33.3 | 1.84 | 5.53 | 2.84 | 8.86 | 1.230 | 45.0 |
| | 7 | 32.0 | 2.24 | 7.28 | 3.2 | 9.92 | 1.635 | 51.0 |
| | 9 | 20.6 | 1.241 | 6.02 | 2.04 | 9.90 | 1.179 | 54.5 |
| Weight, 9.10 to 9.00 kilos. | | | | | | | | |
| 7. Diet: meat, 143 g.; suet, 72 g. per diem (average)—13 days. | | | | | | | | |
| Jan. | 18 | 27.2 | 1.104 | 4.06 | 3.56 | 13.16 | 2.086 | 58.3 |
| | 22 | 26.0 | 1.40 | 5.67 | 8.03 | 30.8 | 5.096 | 63.4 |
| | 25 | 20.0 | 1.176 | 5.88 | 5.08 | 28.8 | 3.692 | 64.0 |
| | 30 | 26.0 | 1.456 | 5.60 | 6.55 | 25.2 | 3.640 | 57.0 |
| Weight, 9.9 to 9.00 kilos. | | | | | | | | |

TABLE I—*Continued*

| Date | Dry wt. of Faeces g. | Total N | % N | Total fat | % fat | Total fatty acid | % fatty acid of total P. E. Extract |
|---|-------------------------------|---------|------|-----------|-------|------------------------|---|
| 8. Diet: caseinogen, 60 g.; palmine, 30 g. per diem for 7 days. | | | | | | | |
| Mar. 11 | 16.5 | 0.880 | 5.32 | 1.73 | 10.1 | 1.218 | 73.1 |
| 13 | 12.1 | 0.545 | 4.55 | 2.34 | 19.3 | 1.224 | 52.8 |
| 14 | 10.3 | 0.462 | 4.48 | 1.09 | 10.56 | 0.789 | 74.0 |
| 15 | 11.0 | 0.531 | 4.83 | 0.891 | 8.10 | 0.625 | 70.2 |
| 16 | 10.65 | 0.522 | 4.90 | 0.880 | 8.12 | 0.667 | 77.7 |
| 18 | 21.6 | 0.937 | 4.34 | 2.268 | 11.08 | 1.944 | 86.0 |

Weight, 8.90 to 8.75 kilos.

| | | | | | | | |
|---|------|-------|------|-------|-------|-------|------|
| 9. Diet: caseinogen, 60 g.; cod liver oil, 40 c.c. (17th–26th). | | | | | | | |
| Mar. 19 | 11.6 | 0.544 | 4.90 | 0.673 | 6.06 | 0.395 | 58.6 |
| 21 | 9.7 | 0.489 | 5.04 | 0.440 | 4.46 | 0.275 | 61.6 |
| 23 | 12.0 | 0.554 | 4.62 | 0.430 | 3.58 | 0.307 | 71.5 |
| 24 | 13.8 | 0.656 | 4.76 | 0.620 | 4.52 | 0.391 | 63.1 |
| 25 | 9.8 | 0.384 | 3.92 | 0.637 | 6.78 | 0.445 | 69.9 |
| 26 | 15.3 | 0.686 | 4.48 | 0.921 | 6.32 | 0.782 | 25.0 |
| 29 | 15.0 | 0.609 | 4.06 | 1.65 | 11.08 | 0.879 | 53.3 |

27th and 28th—Butter fat, 40 g. per diem.

Weight, 8.75 to 8.78 kilos.

Utilisation of N.

No. 1 shows results upon an exclusive diet of 200 g. of puppy biscuit, given over a period of five days; the utilisation of the N is 93.8 per cent. of the total ingested. On a diet of cooked meat the total N excreted falls, and as the results in No. 2 show, the protein of meat is readily dealt with by the normal gastric and intestinal secretions, only 3.5 per cent. being lost by the faeces. On a mixed diet of meat, milk and puppy biscuit (No. 3), the total daily excretion of faecal N rises and remains steady at about 1 gram. The absorption figure of 89.71 per cent. is probably lower because of the large amount of starch in puppy biscuit which is available for the immediate supply of energy.

No. 4 shows the effect of a milk diet continued for a period of nine days. During the first two days 700 cc. of milk were ingested, and on the third day of the period the first estimation of N gives a remarkably uniform figure. In the course of the last six days of the experiment 2400 cc. of milk and 448 g. of puppy biscuit were given, equal to 25.44 g. N, of which 3.376 g. were found in the faeces, giving an absorption of 92.70 per cent. For the whole period the utilisation figure for N was 87.2 per cent.

In No. 5, where the diet consisted of 400 cc. of milk with puppy biscuit and lemco, the latter in quantities of from 2–30 g. per diem, the percentage

N excreted daily is not appreciably altered, but here the utilisation is very good, being 97.27 per cent. This of course may be due to the appetising effect of the added lemco; at any rate it shows that given a good appetite and milk not in excess of the needs of the organism, the protein, fat and carbohydrate of milk will be practically all assimilated.

The protein of milk is well utilised and even the addition to the diet of concentrated protein preparations does not appreciably alter the daily loss of N. The results, as shown in No. 6, where ereptone was added to a diet of meat sufficient in itself, clearly demonstrate the avidity and the ease with which the organism deals with protein. A loss of only 5.28 g. of N on such a nitrogenous diet is remarkable.

A diet of meat and mutton fat given over a period of nineteen days resulted in a slight increase in the dry bulk of the faeces which could be accounted for by the nature of the diet and the longer period between the days of defaecation. As No. 7 indicates, the daily N is slightly increased, but the percentage is within the usual normal variations. The percentage utilisation of N is 96.2.

On a diet of caseinogen and palmine (No. 8) the absorption of N is 95.37 per cent. The addition of cod liver oil to the diet had practically no effect upon N absorption, the percentage N utilisation in No. 9 being 96.5.

The N of the faeces.

It is known that the composition of the faeces is not greatly altered by feeding dogs on any of the simple food stuffs. This does not hold good for herbivora. Fritz Voit [1892] by his loop experiments showed that the excretions from the isolated gut were similar in constitution to that obtaining in the whole gut. The N content was almost equal in both as calculated per square metre per 24 hours. The loop however contained a greater amount of fat and fatty acids. From these experiments he maintained that the N of the faeces was largely derived from substances excreted through the wall of the intestine and was therefore a product of protein metabolism.

In my tables it is seen that the N excretion remains fairly constant on any diet which does not consist exclusively of meat, for on such a pure meat diet the N excretion is reduced from the approximate daily loss of 1 g. to 0.5 g. per day, the utilisation varying from 89.71 per cent. to 96.53 per cent.

With milk the N figure averages 1.1 g. per day, but the addition of carbohydrate raises the daily N loss to 1.5 g. per day. Where vegetables

are added to a large milk diet the N loss is evident, but whether this increase is due to an increased secretory activity or less complete utilisation of N, it is difficult to say. As has been pointed out in Nos. 4 and 5, milk, when given in normal amounts, is best assimilated when such a palatable article of diet as puppy biscuit is added. As with meat so with all concentrated protein foods, the N loss is small, the percentage daily loss varying from 3.50 to 5.28 g., which is equivalent to a total daily loss of from 0.38 to 0.71 g. On a low N diet the utilisation was 88 per cent., while on a high N diet the utilisation averaged 95.89 per cent.

Utilisation of fat.

On a mixed diet the fat absorption varies from 89 to 96 per cent., and depends upon the form in which the fat is ingested. On a diet of puppy biscuit the fat absorption was 94.98 per cent., while on a diet of meat only (No. 2) the absorption was 87.1 per cent. In this latter case the fatty acids recovered from the faeces amounted to 7.6 per cent. of total fat in diet, which is equal to a percentage utilisation of pure fat, estimated as fatty acid, of 92.4. An increase of fat in the diet leads to an increased percentage utilisation, as shown by the results in No. 3, where the absorption figures for fat as total petroleum ether extract and pure fat are 92.98 per cent. and 95.86 per cent. respectively.

In No. 4, where the diet consisted largely of milk, 87.68 g. of fat were given during the last six days of the experiment, of which 5.254 g. were lost, which is equal to a utilisation of 94.0 per cent. For the total period of the experiment the absorption of total fat amounted to 91.82 per cent. and of pure fat to 95.34 per cent., proving as has been done so often that a natural emulsion of fat such as obtains in milk is the best condition for its assimilation. On a mixed diet of milk, puppy biscuit and lemco excellent utilisation figures were obtained, as the results in No. 5 show. The amount of fatty acid recovered from the faeces was 2 per cent. of the total fat given, while the total fat extracted amounted to 3.64 per cent. of the fat ingested.

Mutton fat, which has a melting point of from 49° to 51°, is well utilised even when given in large quantities of 50 to 90 g. a day. This confirms von Noorden's statement that a larger quota of fat in the diet effects a more complete absorption. Here 807.86 g. of fat were given during the period under observation; the meat in the diet accounted for 85.6 g. of fat, making a total of 893.46 g. of fat, of which 23.94 g. were excreted, giving a utilisation of 97.4 per cent. The fatty acid recovered from the faeces amounted to

1.79 g., which is equivalent to a pure fat utilisation of 98.21 per cent. Munk [1884], feeding dogs with suet alone, obtained results indicating an absorption of 90 to 94 per cent. of fat.

Stearin and palmitin are the chief constituents of several preparations which are made to take the place of butter, such as margarine and palmine, etc. The stearin of these preparations is extracted from "kernel" palm nut oil after the removal of the more liquid portion by hydraulic pressure. The melting point of the palm nut oil is 34° – 40° , its chief constituent being palmitin. The small amount of stearin in palmine gives to it that solid consistency which makes it such an ideal preparation for feeding purposes.

During the experiment—No. 8—204.0 g. palmine were ingested and 9.199 g. of fat excreted, which is an absorption of 95.5 per cent.; the pure fat utilisation was 95.6 per cent.

No. 9 shows that the free fatty acid in cod liver oil facilitates the emulsifying and the subsequent absorption of the oil; 360 g. of the oil were given and 3.721 g. of fat and fatty acid were excreted; utilisation, 98.97 per cent. A point of note here is that the percentage fatty acid of total fat extracted is distinctly higher than in the preceding tables, and estimating the utilisation from the point of total fatty acid recovered we find almost a total assimilation, 99.04 per cent.

On a low fat diet the utilisation was 87.1 per cent. and on a high fat diet 97.4–98.9 per cent.

The effect of pancreas feeding.

It could not be expected that the addition of pancreas to the diet in normal dogs would materially improve such absorption figures as 97.27 per cent., etc., which are the result of feeding on a mixed diet composed largely of meat, with the addition of puppy biscuit and a bone, ingredients calculated to bring into play to their fullest extent not only the physical but the psychical factors in digestion.

A dog fed on 250 g. of meat and 150 g. of pancreas for three days excreted at the end of that period 1.84 g. N (5.74 per cent.) and 1.92 g. fat (6.25 per cent.), which is equal to a utilisation of N, 96.15 per cent. and of fat, 97.15 per cent.

UTILISATION OF PROTEIN N AND FAT IN DIABETIC ANIMALS.

A. In partially depancreatized dogs.

Condition of animals. The day after operation Dog I, which had a graft of pancreatic gland in its abdominal wall, weighed 8.6 kilos and excreted

1.85 g. sugar in the 24 hours sample of urine. On the following day the loss of sugar had risen to 30 g., about which figure it remained for a week, after which for a few days the excretion rose to between 40 and 50 g., subsequently falling to 28 g. and remaining with varying fluctuations at about 30 g. per day till the eighteenth day after operation, upon which day the dog was killed by bleeding under CHCl_3 anaesthesia. The D : N ratio in this dog varied from 3.8 : 1 on the third day to 5.4 : 1, which latter figure was reached on the tenth day after operation, the ratio for the following days being between 3.2 : 1 and 5.0 : 1. From these data one sees that despite the graft of pancreatic tissue and the apparently good condition of the animal, a decidedly diabetic condition obtained, in view of which the following figures for utilisation are worthy of note. In comparison with the totally depancreatized dogs the slower loss in weight and the higher respiratory quotient—0.69 to 0.740—show that the disturbance was not so acute as in cases of immediate depancreatization.

Dog II, in which a portion of the gland was left *in situ*, weighed 8.0 kilos before operation. On the fourth day after operation the sugar excretion had risen to 5 g. in the 24 hours with a D : N ratio of 1 : 0.7, while on the eighth day the total excretion for the 24 hours was 11.0 g., from which time it rapidly fell until only a trace of sugar upon occasional days could be obtained. The loss of weight was very gradual, the respiratory quotient remained normal 0.78 to 1.03 according to diet, and the glycosuria, up to the time the dog was totally depancreatized, was transient.

Utilisation of N. Dog I. In Nos. 10 and 11 (Table II) the N absorption is 93.2 and 95.4 per cent. Pancreas was added to the diet in both cases;

TABLE II.

Partially depancreatized Dogs.

10. Dog I. Partially depancreatized; c graft in abd. wall.

Diet: May 21, meat 100 g.; May 22–June 3, meat, 150 g., pancreas, 50 g.; June 3–8, meat, 200 g., pancreas, 50 g.; June 9 and 10, meat, 200 g., pancreas, 60 g.

| Date | Dry wt. of Faeces g. | Total N | % N | Total fat | % fat | Total fatty acid | % fatty acid of total P. E. Extract | Wt. of dog in kilos |
|--------|-------------------------------|---------|------|-----------|-------|------------------------|---|---------------------------|
| May 23 | 44.0 | 2.834 | 6.44 | 13.53 | 30.76 | 10.34 | 76.6 | 8.1 |
| 26 | 14.1 | 0.852 | 6.04 | 4.935 | 33.76 | 3.567 | 70.47 | 7.60 |
| June 2 | 35.0 | 2.264 | 6.44 | 10.50 | 30.10 | 7.154 | 72.7 | 6.80 |
| 6 | 27.0 | 1.890 | 7.0 | 4.320 | 16.56 | 3.604 | 80.9 | 6.60 |
| 7 | 26.0 | 1.929 | 7.42 | 3.640 | 14.0 | 2.496 | 69.0 | 6.50 |
| 11 | 24.2 | 1.829 | 7.56 | 2.90 | 12.16 | 1.920 | 66.2 | 6.20 |

Weight before op. 9.15 kilos, Op. 18th, Weight 20th, 8.65 kilos.

TABLE II—*Continued.*

| 11. Diet: caseinogen, 60 g.; pancreas, 100 g.—19 days. | | | | | | | | |
|---|-------------------------------|-----------|---------------------------|-----------|--------|------------------------|---|---------------------------|
| Date | Dry wt. of Faeces g. | Total N | % N | Total fat | % fat | Total fatty acid | % fatty acid of total P. E. Extract | Wt. of dog in kilos |
| June 15 | 17.7 | 0.977 | 5.18 | 1.848 | 10.44 | 1.358 | 73.4 | 6.0 |
| 16 | 23.4 | 1.579 | 6.72 | 0.799 | 3.36 | — | — | 5.75 |
| 17 | 24.0 | 1.310 | 5.46 | 2.112 | 8.88 | 1.632 | 77.2 | 5.70 |
| 19 | 21.4 | 1.02 | 4.76 | 1.914 | 8.94 | 1.519 | 79.4 | 5.60 |
| 20 | 28.2 | 1.421 | 5.04 | 2.989 | 9.68 | 1.762 | 61.9 | 5.50 |
| 22 | 36.4 | 1.783 | 4.90 | 4.659 | 12.20 | 3.101 | 69.8 | 5.45 |
| 23 | 8.0 | 0.3584 | 4.48 | 1.280 | 15.76 | 1.20 | 94.0 | 5.25 |
| 26 | 23.3 | 0.9786 | 4.20 | 6.291 | 26.82 | 5.545 | 87.7 | 5.10 |
| 12. Dog II. Portion of gland left <i>in situ</i> ; c pancreatic duct intact. | | | | | | | | |
| Diet: meat, 100 g.; pancreas, 50 g.; May 21–June 3. Meat, 150 g.; pancreas, 50 g.; June 4–10. | | | | | | | | |
| | Weight, 8.0 kilos. | Op. 18th. | Weight, 20th, 7.75 kilos. | | | | | |
| May 22 | 30.5 | 0.818 | 9.24 | 5.155 | 16.98 | 4.575 | 85.1 | 7.40 |
| 24 | 12.4 | 1.160 | 9.36 | 1.860 | 15.5 | 1.50 | 80.7 | 7.20 |
| 26 | 27.0 | 2.419 | 8.96 | 5.94 | 22.0 | — | — | 7.00 |
| 27 | 40.0 | 3.080 | 7.70 | 4.40 | 11.4 | 3.290 | 74.8 | 6.90 |
| 28 | 9.0 | 0.680 | 7.56 | 1.681 | 18.68 | 1.07 | 71.0 | 6.80 |
| 31 | 8.5 | 0.643 | 7.56 | 1.275 | 15.12 | 1.013 | 78.8 | 6.80 |
| June 1 | 35.0 | 2.264 | 6.44 | 10.5 | 28.12 | 7.154 | 72.7 | 6.75 |
| 2 | 5.6 | 0.450 | 7.98 | 0.622 | 11.1 | 0.397 | 65.0 | 6.75 |
| 3 | 9.2 | 0.834 | 6.44 | 1.454 | 15.1 | 1.097 | 75.4 | 6.75 |
| 4 | 11.5 | 0.744 | 6.86 | 1.242 | 10.88 | 0.654 | 53.0 | 6.85 |
| 6 | 16.8 | 1.15 | 8.40 | 1.405 | 8.36 | 0.814 | 60.3 | 7.00 |
| 8 | 11.0 | 0.924 | 7.70 | 1.430 | 12.381 | 0.782 | 59.4 | 6.75 |
| 10 | 20.5 | 1.578 | 8.96 | 3.895 | 18.68 | 2.844 | 70.9 | — |
| 11 | 12.6 | 1.130 | 7.98 | 1.385 | 10.94 | 0.894 | 71.0 | 6.75 |
| 13. Diet: meat, 150 g.; pancreas, 30 g. (16th to 20th). | | | | | | | | |
| 17 | 8.5 | 0.607 | 7.14 | 0.822 | 9.66 | 0.434 | 55.5 | 6.75 |
| 18 | 13.55 | 1.021 | 7.564 | 1.49 | 10.90 | 1.035 | 66.7 | — |
| 20 | 11.3 | 0.886 | 7.84 | 1.34 | 11.04 | 0.7063 | 53.0 | 6.75 |
| 14. Diet: meat, 250 g.; pancreas, 50 g.—5 days. | | | | | | | | |
| 13 | 21.5 | 1.535 | 7.14 | 3.775 | 17.56 | 1.587 | 42.0 | 6.35 |
| 14 | 18.0 | 1.285 | 7.14 | 2.660 | 14.80 | 1.89 | 74.6 | 6.40 |
| 16 | 16.7 | 1.403 | 8.40 | 2.348 | 14.06 | 0.902 | 40.0 | 6.50 |
| 15. Diet: June 30, meat, 100 g., puppy biscuit, 100 g. Thyroid, July 2–4, 1 g.; 5–6, 1.5 g.; 7–8, 2 g.; 9–10, 3 g. | | | | | | | | |
| July 1 | — | 0.916 | — | 0.891 | — | 0.810 | 61.4 | — |
| 2 | 23.6 | 1.784 | 7.56 | 2.025 | 8.58 | 1.376 | 68.2 | 6.60 |
| 3 | 17.0 | 1.142 | 6.72 | 0.8262 | 4.86 | 0.435 | 52.6 | 6.60 |
| 4 | 30.0 | 2.058 | 6.86 | 1.764 | 5.88 | 0.936 | 55.0 | — |
| 5 | 31.2 | 2.184 | 7.0 | 1.50 | 4.84 | 0.775 | 52.8 | 6.62 |
| 6 | 17.0 | 1.071 | 6.3 | 1.221 | 7.10 | 0.675 | 56.8 | — |
| 7 | 26.0 | 1.310 | 5.04 | 1.70 | 6.54 | 1.107 | 65.1 | 6.65 |
| 8 | 39.2 | 2.566 | 6.58 | 2.74 | 7.0 | 1.113 | 41.0 | 6.60 |
| 9 | 32.5 | 2.138 | 6.58 | 2.35 | 7.20 | 1.170 | 50.0 | 6.50 |
| 10 | 24.7 | 1.729 | 7.0 | 2.021 | 8.18 | 1.052 | 52.1 | — |
| 11 | 35.8 | 1.356 | 6.30 | 2.899 | 8.10 | 1.525 | 52.6 | 6.40 |

in the former the chief constituent was meat, 150–200 g. per day, in the latter, caseinogen, 60 g. per day. Both these experiments show a remarkable utilisation of N, the protein being presented in a most assimilable form.

Dog II. The figures in No. 12 are the result of a diet similar to, but more generous than, the preceding, and the observation was continued over a much longer period—three weeks. The excretion of N is less; during the period 2490 g. of meat and 890 g. of pancreas were given, which is equal to 102.09 g. and 26.7 g. N respectively, making a total of 128.79 g. N, of which 19.87 g. were excreted, giving a utilisation of 86.0 per cent. The average daily excretion, which was 0.990 g. N, is quite a normal figure.

In No. 13, where the same diet was repeated, there was an improved N utilisation, 91.7 per cent. An increased meat diet with pancreas still maintains an improved absorption, as is shown by No. 14, where the N utilisation was 93.27 per cent.

These high figures can be due to no other cause than the beneficial effect exerted by the added pancreas in the diets, because on a mixed diet of meat and puppy biscuit, which in normal animals leads to improved protein absorption, and regardless of the fact that the protein need of the organism was accentuated by thyroid administered, the digestive system did not on this mixed diet, without pancreas, maintain its previous powers of utilisation, the N absorption falling to 79.86 per cent. (No. 15.)

It is seen that where a portion of the gland is left *in situ* the results are similar to those obtained with a graft of the gland. Most of the previous work has been done irrespective of the addition of pancreas, and in these experiments where no pancreas has been added to the diet, the N loss has varied from 17 to 27.5 per cent.

Utilisation of fat. In Dog I the amount of fat ingested was 209 g. and that excreted 39.82 g., which gives a utilisation of 80.95 and of pure fat 86.1 per cent. The first sample of the experiment contained a large amount of fat, 13.53 g. The second, three days after, which was of much smaller bulk, contained 4.935 g. The percentage of fat in both samples was over 30. These figures are high because of the constipation existing, but with a more regular action of the intestine the total amount of fat per sample approximates more closely to a normal figure, but that fat is being lost is quite evident both from the total and percentage figures. In No. 12 almost the same amount of fat was given, there being double the daily amount of pancreas ingested as in the previous case. Here the absorption figure has improved, amounting to 87.44 per cent. of total fat and 91.7 per cent. of pure

fat, calculated as fatty acid. While it is possible to get a good absorption of fat, it cannot be maintained for long periods; as can be readily understood, a diabetic organism cannot be expected to show the same adaptability as the normal.

Dog II was fed for three weeks on a meat and pancreas diet. The fat loss was comparatively large, being 23·9 per cent., a figure which is more in agreement with Weintraud's results, but in view of better absorption on other occasions cannot be accepted as being an indication of the failure of the diabetic to absorb fat to any great extent.

On a rather sparing diet of meat and pancreas the amount of fat given was 30 g., of which 3·65 g. were excreted, being equal to a utilisation of 87·83 per cent. estimated as total fat absorbed and of 92·75 per cent. as pure fat. With a more liberal diet similar to the foregoing the utilisation was practically the same, viz. 85·95 per cent. and 93 per cent. (No. 14.)

With partially depancreatized dogs on a diet of meat and pancreas there is an average loss of fat amounting to 14·37 per cent.

On a diet of meat and puppy biscuit without the addition of pancreas, the utilisation of the small amount of fat in the diet is very poor (No. 15), the figures being, for total fat, 72·12 per cent., and for pure fat, 84·7 per cent. A loss of 27·8 per cent. of fat on such a diet should never occur when pancreas is added. In all these cases of partial depancreatisation the excretion of fatty acid with pancreas feeding is 60–80 per cent. of the total petroleum ether extract. The lack of pancreas in the diet results in a lowering of the percentage excretion of the fatty acid to between 40 and 65. •

B. *In totally depancreatized dogs.*

Condition of the animals. As several dogs having a graft of pancreas in their abdominal wall or a piece left *in situ* have been kept in this laboratory in good condition for over two months, this preliminary operation, leading to a mild type of diabetes, undoubtedly allows the animal to adapt itself to the new conditions and so lessens the shock of the subsequent total extirpation; it also helps the animal to conserve its energy for a longer time during the much more severe type of diabetes produced by the second operation.

In totally depancreatized dogs there is always some evidence which at first hand leads one to suspect an increased amount of fat in the faeces. On a diet of meat there is an increased faecal odour, due to protein decomposition in the large intestine, and with a diet of fat there is invariably a typically

fatty foul-smelling stool, which after drying on glass plates presents the appearance of sheets of dark brown gelatin. It is evident therefore that the digestive and absorptive powers will vary according as the dog has been totally depancreatized by two stages or at one operation, because of the sudden onset of severe diabetes and the worse condition of the dog after immediate depancreatization.

TABLE III.

Totally depancreatized Dogs.

16. Dog III. Total depancreatization.

Diet: July 19, meat, 100 g., pancreas, 50 g.; July 22, meat, 200 g., pancreas, 100 g.

| Date | Dry wt. of Faeces g. | Total N | % N | Total fat | % fat | Total fatty acid | % fatty acid of total P. E. Extract | Weight of dog in kilos |
|---------|-------------------------------|---------|------|-----------|-------|------------------------|---|------------------------------|
| July 21 | 14.6 | 1.242 | 7.70 | 4.322 | 29.6 | 2.073 | 48.0 | 5.80 |
| 22 | 15.6 | 1.267 | 8.12 | 2.833 | 18.16 | 2.037 | 71.9 | 5.80 |
| 23 | 9.3 | 0.890 | 9.52 | 1.55 | 16.66 | 1.109 | 71.6 | 5.70 |
| 24 | 11.7 | 0.982 | 8.40 | 2.14 | 18.28 | 1.183 | 55.0 | 5.65 |
| 25-26 | 28.1 | 2.469 | 8.82 | 4.158 | 14.80 | 2.154 | 57.8 | 5.60 |
| 27 | 10.3 | 0.894 | 8.68 | 1.456 | 14.14 | 0.731 | 50.2 | 5.50 |
| 28 | 11.1 | 0.8081 | 7.28 | 2.775 | 25.0 | 1.607 | 57.9 | 5.35 |
| 30 | 14.6 | 0.930 | 6.44 | 3.35 | 23.10 | 1.989 | 59.0 | 5.20 |

Before op. 6.5 kilos. Op. July 17. Weight, July 19, 6.15 kilos.

17. Dog IV. Totally depancreatized.

Diet: Feb. 1, meat, 500 g. for over 3 days.

| | | | | | | | | |
|--------|------|-------|------|-------|-------|-------|------|-----|
| Feb. 3 | 34.5 | 3.236 | 9.38 | 8.280 | 23.90 | 7.445 | 89.6 | 6.8 |
| 4 | 16.5 | 1.60 | 9.94 | 3.30 | 20.44 | 2.673 | 81.0 | 5.6 |

Op. Jan. 30; weight before, 7.0 kilos; Feb. 1, 6.35 kilos.

18. Diet: meat, 160 g.; pancreas, 50 g.; milk, 50 g.—5 days.

| | | | | | | | | |
|----|------|-------|------|-------|--------|-------|------|------|
| 21 | 18.0 | 1.323 | 7.35 | 3.240 | 18.46 | 2.45 | 75.7 | 6.75 |
| 22 | 11.2 | 0.909 | 8.12 | 1.789 | 17.10 | 1.590 | 83.0 | 6.50 |
| 23 | 14.6 | 1.267 | 8.68 | 1.752 | 12.051 | 1.452 | 80.0 | 6.40 |

After op. 16th, 7.2 kilos.

19. Diet: meat, 600 g.; pancreas, 100 g. (i.e. total).

| | | | | | | | | |
|--------|------|-------|------|-------|-------|-------|------|-----|
| Mar. 5 | 12.6 | 1.12 | 9.73 | 2.06 | 15.37 | 1.431 | 70.0 | 7.6 |
| 6 | 24.4 | 1.133 | 4.76 | 4.88 | 19.8 | 3.618 | 74.1 | 7.3 |
| 7 | 24.8 | 2.15 | 8.68 | 4.037 | 16.28 | 2.536 | 63.5 | 6.7 |

Op. Mar. 2. Weight before op. 8.0 kilos; after Mar. 4, 7.5 kilos.

Dog III from which the results in No. 16 (Table III) were obtained was made totally diabetic by the two-stage operation, and a marked glycosuria immediately supervened upon the second operation, the dog eliminating 80 to 100 per cent. of any sugar given to it. The D : N ratio varied between 2.8 and 3.1 : 1, and the total excretion of sugar for 24 hours was between

30 and 40 g. The loss of weight was at about the rate of one kilo in ten days, which is similar to what obtained in the first partially depancreatized dog. Another sign that a distinct metabolic disturbance had ensued was the low level of the respiratory quotient, the rise of which consequent upon sugar injection was greatly diminished. Dog IV was the subject of severe diabetes, losing weight rapidly and excreting large quantities of sugar. This dog as well as the two following (Nos. 18 and 19) were totally depancreatized at one operation, and, as will be pointed out later, received no pancreas in their diet. The others, though not wasting so rapidly as the former, lost flesh somewhat more quickly than those dogs which were only partially depancreatized. They had a D : N ratio of 3·8 : 1 and upwards, excreted 15 to 50 g. of sugar per diem, and eliminated 85–100 per cent. of sugar added to their diet.

Utilisation of N. The results obtained from a dog which had its pancreas removed in a two-stage operation are given in No. 16. The diet consisted of meat and pancreas and was in excess of the absolute requirements of the organism. The N utilisation here was 92·1 per cent., which is even within the limits of the absorption of normal animals. The following experiment shows very clearly what a detrimental effect immediate depancreatisation has upon the digestive functions of an animal. No pancreas was added to the diet. There was a loss of 21·9 per cent. N given in the food. In contrast to this, No. 18 shows how effective is the addition of pancreas. Over a period of five days 44·0 g. N were given, of which 3·499 g. were recovered in the faeces, which amounted to a utilisation of 92·0 per cent.

It is evident that for good absorption the dog must make a good recovery after the operation. The absorption shown in No. 19 is very poor, due doubtless to the fact that the dog was not in good condition, for this dog showed signs of gastric and intestinal disturbances on the fourth day after operation and died suddenly on the evening of the fifth. The amount of protein ingested as meat was approximately 500 g. and 100 g. of pancreas, equal to 25·05 g. N. 4·413 g. were excreted, giving a utilisation of 82·43 per cent.

Utilisation of fat. The absorption of fat in a dog made diabetic by the two-stage operation was very good, falling very little below the best obtained with a graft of the pancreatic gland. The amount of pure fat recovered from the faeces in this case was 10·06 g., which gives a percentage absorption of 89·94, while for total fat estimated the utilisation figure was 82·7 per cent.

The results of the following experiment (No. 17) are typical of the

far-reaching disturbance of fat digestion and assimilation consequent upon immediate total depancreatization, when no pancreas is administered with the food. The total fat recovered was 67.4 per cent. of the amount given, being equal to 32.6 per cent. utilisation.

That pancreas influences fat assimilation to a marked degree is shown by the results in No. 18, where the utilisation figures for total fat and pure fat were 88.3 per cent. and 90.5 per cent. respectively. Once digestive disturbances have set in, all absorption will become deranged, no matter what dietetic means be taken to improve it, e.g. the addition of puppy biscuit or pancreas: this is shown by the poor fat absorption figure of 69.8 per cent. (No. 19.)

DISCUSSION.

These results indicate that the removal of the pancreas causes an immediate and serious disturbance of the digestive functions as regards both protein and fat, and also, as the conditions of these animals show, the power of oxidising glucose is more or less destroyed according to the degree of the diabetes produced.

We see from these results that the figures for utilisation in cases of total depancreatization with pancreas feeding are somewhat better than in those of partial depancreatization without pancreas. Still in the total diabetic the function of the absent pancreatic juice must be carried on by other secretions, gastric and succus entericus, as we have no knowledge of any deterrent effect upon the secretions of other juices. Moorhouse [1915] in a recent publication shows that in partially depancreatized dogs there may be an increase of metabolism amounting to 9 per cent., while in total depancreatization such an increase may vary from 9 to 38 per cent. of the normal. He holds that this increase of metabolism runs parallel to the severity of the case and that it is an expression primarily of interference with carbohydrate utilisation and secondarily with protein and perhaps fat metabolism. The protein destruction is enormously increased, while the actual part taken by protein in the oxidations, i.e. the energy distribution of the proteins, is only slightly increased, 5 to 10 per cent. This increased metabolism would explain the good percentage utilisation of protein and fat in the attempt to make good the great expenditure, when the power of sugar utilisation is more or less lost.

CONCLUSIONS.

1. In normal dogs the N utilisation on a high protein diet varied from 95 to 96.5 per cent., while on a low protein diet it was 88 per cent.

2. Fat utilisation on a high fat diet was 97 to 98.9 per cent., on a low diet 87.1 per cent.

3. In partially depancreatized animals there is very little difference in power of assimilation, whether the piece of gland is grafted in the abdominal wall or left *in situ*, but the addition of pancreas to the diet exerts a most favourable effect in both cases.

Utilisation figures:

| | | | |
|-----------------------------------|-------|----------------------------------|--------------------|
| With part of gland grafted | .. | N = 93-95.4 % Fat = 80-87.4 % | } With pancreas |
| With part of gland <i>in situ</i> | .. | N = 86-93.27 % Fat = 76-88 % | |
| Without pancreas | | N = 79.86 % Fat = 72.12 % | |

4. Total depancreatization leads to an immediate and severe diabetes which is usually terminal in about a week or ten days. This condition is alleviated by performing the operation in two stages, the first being a partial depancreatization.

5. The addition of pancreas to the food is as beneficial in one-stage operations as it is in the two-stage operation.

6. Immediate depancreatization without pancreas in the diet produces grave disturbances of digestion and absorption, resulting in a loss of 22 per cent. of N and 67.4 per cent. of fat. The addition of pancreas is most beneficial, the utilisation of N rising to 92 per cent. and of fat to 70-88 per cent.

7. The deleterious effects of extirpation of the pancreas on fat absorption and nitrogen metabolism are thus by no means so serious as has been found by other observers quoted in the introduction to this paper.

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